AMENDMENT

In the Specification:

Please insert the attached "Sequence Listing" as separately numbered pages 1-14 after the abstract, replacing all previously provided sequence listings.

Please replace the title with the following rewritten title:

Nucleic Acids Encoding Non Aggregating Fluorescent Proteins and Method For Using Same

Please replace the Abstract with the following rewritten Abstract:

Nucleic acid compositions encoding non-aggregating chromo/fluoroproteins and mutants thereof, as well as the **encoded** proteins ~~encoded by the same~~, are provided. The proteins of interest are polypeptides that are non-aggregating colored and/or fluorescent proteins, where the ~~the~~ non-aggregating feature arises from the modulation of residues in the N-terminus of the protein and the chromo and/or fluorescent feature arises from the interaction of two or more residues of the protein. Also provided are fragments of the subject nucleic acids and the peptides encoded thereby, as well as antibodies to the subject proteins and transgenic cells and organisms. The subject protein and nucleic acid compositions find use in a variety of different applications. Finally, kits for use in such applications, e.g., that include the subject nucleic acid compositions, are provided.

Please replace the paragraph beginning on line page 6, line 31 with the following rewritten paragraph:

Nucleic acid compositions encoding non-aggregating chromo/fluoroproteins and mutants thereof, as well as the **encoded** proteins ~~encoding the same~~, are provided. The proteins of interest are non-aggregating proteins that are colored and/or fluorescent, where the non-aggregating feature arises from the modulation of residues in the N-terminus of the proteins and the chromo and/or fluorescent feature arises from the interaction of two or more residues of the protein. Also of interest are proteins that are substantially similar to, or mutants of, the above specific proteins. Also provided are fragments of the nucleic acids and the peptides encoded thereby, as well as antibodies to the subject proteins, and transgenic cells and organisms that include the subject nucleic acid/protein compositions. The subject protein and nucleic acid compositions find use in a variety of different applications. Finally, kits for use in such applications, e.g., that include the subject nucleic acid compositions, are provided.

Please replace the paragraph beginning on page 38, line 14 with the following rewritten paragraph:

Site-directed mutagenesis was performed by PCR with primers containing appropriate target substitutions. All mutants were cloned between *Bam*HI and *Hind*III restriction sites of the pQE30 vector (Qiagen). Recombinant proteins were 6 × Histidine-tagged to contain the sequence 'MRHHHHHHGS' **(SEQ ID NO: 31)** instead of the first Met. After overnight expression in *E. coli*, fluorescent proteins were purified using TALON Metal Affinity Resin (CLONTECH). SDS-PAGE analyses revealed that proteins were at least 95% pure.

Please replace the paragraph beginning on page 33, line 15 with the following rewritten paragraph:

The detection of cell-bound fluorescence is typically performed using a flow cytometer, such as a ~~FACSVantage™, FACSVantage™ SE, or FACSCalibur~~ **FACSVANTAGE™, FACSVANTAGE™ SE, or FACSCALIBUR™** flow cytometer (Becton Dickinson Immunocytometry Systems, San Jose, CA, USA). The lasers chosen for excitation will be determined by the absorption spectrum of the multimeric complex and of any additional fluorophores desired to be detected concurrently in the sample. For example, if the multimeric complex has the spectral characteristics of native DsRed — *e.g.*, a homotetramer in which the fusion protein subunits all have the native DsRed GFP-like chromophore — a standard argon ion laser with 488 nm line can be used for excitation. For detection, the filter sets and detector types will be chosen according to the emission spectrum of the multimeric complex and of any additional fluorophores desired to be detected in the sample. For example, if the multimeric complex has the spectral characteristics of native DsRed, with emission maximum at about 583 nm, fluorescence emission from the complex can be detected in the FL2 channel using a PE setup.

Please replace the paragraph beginning on page 34, line 3 with the following rewritten paragraph:

The T lymphocyte-containing sample can be a whole blood sample, typically a peripheral venous blood specimen drawn directly into an anticoagulant collection tube (*e.g.*, EDTA-containing or heparin-containing ~~Vacutainer™~~ **VACUTAINER™** tube, Becton Dickinson ~~Vacutainer~~ **VACUTAINER™** Systems, Franklin Lakes, NJ, USA).

Please replace the paragraph beginning on page 34, line 7 with the following rewritten paragraph:

Advantageously, the T lymphocyte-containing sample can also be a whole blood sample that has been treated before detection with a red blood cell (RBC) lysing agent as is described, *inter alia*, in Chang *et al.*, U.S. Patent Nos. 4,902,613 and 4,654,312; lysing agents are well known in the art and are available commercially from a number of vendors (FACS™ Lysing Solution, Becton Dickinson Immunocytometry Systems, San Jose, CA, USA; ~~Cal-Lyse™~~ **CAL-LYSE™** Lysing Solution, Caltag Labs, Burlingame, CA, USA; No-Wash Lysing Solution, Beckman Coulter, Inc., Fullerton, CA). The sample can optionally be washed after RBC lysis and before detection.

Please replace the paragraph beginning on page 34, line 15 with the following rewritten paragraph:

The sample within which T lymphocytes are desired to be detected according to the methods of the present invention can also be a peripheral blood fraction, advantageously a mononuclear cell (PBMC) fraction. PBMCs can be prepared according to any of the well-known art-accepted techniques, including centrifugation through Ficoll-Paque (Amersham Pharmacia Biotech, Piscataway, NJ, USA) and centrifugation directly in a cell preparation blood collection tube (~~Vacutainer™~~ **VACUTAINER™** CPT™ Cell Preparation Tube, Becton Dickinson, Franklin Lakes, NJ, USA).

Please replace the paragraph beginning on page 35, line 22 with the following rewritten paragraph:

Advantageously, the antibodies used in the methods of the present invention will be prior-conjugated directly to a fluorophore, typically a fluorophore whose emission is

flow cytometrically distinguishable from that of the intrinsically fluorescent multimeric T cell labeling complex and from that of other fluorophores concurrently used in the method. Fluorophores can usefully be selected from the group consisting of fluorescein isothiocyanate (FITC), phycoerythrin (PE), peridinin chlorophyll protein (PerCP), allophycocyanin (APC), Texas Red, ~~Alexa Fluor 488~~ **ALEXA FLUOR® 488** (Molecular Probes, Inc., Eugene Or.), and the tandem fluorophores PerCP-Cy5.5, PE-Cy5, PE-Cy7, and APC-Cy7. Antibodies can also usefully be conjugated to biotin, permitting second stage detection usingfluorophore-labeled streptavidin.

Please replace the paragraph beginning on page 38, line 21 with the following rewritten paragraph:

In greater detail, mutagenesis was performed by the overlap extension method (Ho, S.N., Hunt, H.D., Horton, R.M., Pullen, J.K., Pease, L.R. Site-directed mutagenesis by overlap extension using the polymerase chain reaction. (Gene 1989, 77, 51-59). Briefly, two overlapping fragments of FP coding region were amplified. "Forward cloning" and "reverse mutagenesis" primers were used for 5'-end fragment amplification, and "forward mutagenesis" and "reverse cloning" primers were used for 3'-end fragment amplification. PCR was carried out using ~~Advantage®~~ **ADVANTAGE®** 2 Polymerase Mix (CLONTECH) in 1 × manufacturer's buffer supplemented with 100 μM of each dNTP, 0.2 μM of each primer and 1 ng of plasmid DNA in 25 μl (final volume). The cycling parameters were set at: 95°C for 10 seconds, 65°C for 30 s, 72°C for 30 s. 20 cycles were completed using PTC-200 MJ Research thermocycler. To remove plasmids encoding initial (wild type) protein, the 5'- and 3'-fragments were excised from 2% low-melting agarose gel in 1 ×TAE buffer. To drain the DNA solution, the gel pieces were subjected to 3 freeze-thaw cycles. 5'- and 3'-fragments were combined to obtain full-length cDNA as follows. Equal volumes of 5'-fragment solution, 3'-fragment solution and 3 ×PCR mixture containing ~~Advantage~~ **ADVANTAGE®** 2 Polymerase Mix, buffer and dNTPs were mixed together and subjected to 2-3 cycles of 95°C for 20 s, 65°C for 30 min, 72°C for 30 s. Then, the reaction was diluted 10 fold and 1 μl of the diluted sample was used as a template for PCR with forward and reverse cloning primers (as described above for 5'- and 3'-fragments amplification). As a result, ready-for-cloning fragments containing full-length coding regions with target substitution(s) were generated.

Please replace the paragraph beginning on page 39, line 17 with the following rewritten paragraph:

Selected *E. coli* clones were grown at 37°C in 50 ml to an optical density of (OD) 0.6. At that point, the expression of recombinant FP was induced with 0.2 mM IPTG. The cultures were then incubated overnight. The following day, cells were harvested by centrifugation, resuspended in buffer (20 mM Tris-HCl, pH 8.0; 100 mM NaCl), and disrupted by sonication. Fluorescent proteins were purified from the soluble fraction using ~~TALON~~ **TALON™** Metal Affinity Resin (CLONTECH). Proteins were at least 95% pure according to SDS-PAGE.